DATE: 2/10/72

TO : Dr. Renato Dulbecco

FROM : Dr. Paul Berg

SUBJECT: Attached

Dear Renato:

This is like sending coals to Newcastle. Nevertheless, these are the procedures we have used with the cells I sent you for both growing SV40 and for high efficiency plaque titreing of SV40 DNA.

If there are any problems let me know.

Sincerely,

Paul

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Purification of SV40 as done in Paul Berg's Lab.

Reference: Pagano et al., J. Virol. 7 :635 (1971).

1. 100 confluent plates of MA134 cells in Dulbecco's Modified Eagle's Medium + 10% calf serum + penicillin and streptomycin.

2. infect cells at M.O.i. $\simeq$ 0.01.

3. 3-4 days later, change medium on infected cells.

4. 10 to 11 days post infection, collect cells and medium from plates with help of a rubber policeman.

5. Add 1M Tris, pH 7.6 to cell suspension to give final concentration of 50 mM Tris. This prevents drop in pH during freeze-thawing step.

6. Freeze-thaw cell suspension 3 times using dry ice in ethanol and a 30°C water bath.

7. Dissolve 68 gm solid PEG 6000 in 900 ml lysate to give final concentration of 7% w/v. Stir slowly overnight at 4°C.

8. Centrifuge ppt. at 16,000 xg for 40 min at 4°C.

9. Extract SV40 from pellet by stirring for 12 hrs at 4°C in 85 ml of 0.025 M Tris pH 7.6, 0.15 M NaCl. Centrifuge at 16,000 xg for 40 min at 4°C.

10. Repeat extraction of pellet with 85 ml Tris-NaCl solution.

11. Combined supernatants are layered over a CsCl cushion as follows:

    use 6 SW27 tubes

    a) 3 ml 1.38 gm CsCl/ml
    b) 3 ml 1.30 gm CsCl/ml
    c) 30 ml crude virus preparation

    spin at 25,000 rpm, 3 hrs , at 4°C. Virus is the major band of highest density. Use a suction pipette to remove solution above the virus bands.

12. Dialyze virus against 1 liter 0.025 M Tris, 0.15 M NaCl, 0.001 M $MgCl_2$, pH 7.6 for 12 hrs at 4°C then change dialysate and repeat dialysis.

13. In order to sterilize virus preparation, the final solution is treated with 0.2 ml $CHCl_3$/10 ml virus solution for 10 min at 4°C. Mix $CHCl_3$ by many inversions of tube, rather than vortexing. Centrifuge for 5 min at 4°C (10,000 xg) to separate $CHCl_3$ and collect the virus solution.

14. Adjust the virus to 10% calf serum and freeze in 5 ml aliquots. Final yeild from 100 plates was 50 ml of 2×10^{10} pfu/ml as assayed in CV1-P cells. This is about

$$\frac{50\ (2 \times 10^{10}\ \text{pfu})}{100(6 \times 10^{6}\ \text{cells})} = \frac{1700\ \text{pfu}}{\text{cell}}$$

Titer decreased to 5×10^{9} pfu/ml 3 weeks later.

Procedure for Plaquing SV40 on CV-1 Cells

(S. Kit's procedure as modified by J. Mertz)

The CV-1 line being used was sent to us at passage 236 from Kit's laboratory (called CV-1 P236). The cells are grown in DMEM + 10% Calf Serum + penicillin and streptomycin and transfered using trypsin + EDTA when near confluent by a 10 to 30 fold dilution.

Cells used for plaquing are plated on 60 mm Falcon plastic dishes so that they reach confluency in 3-4 days. (Using one large (80-100 mm) plate of near confluent-to-confluent cells to seed 10-60 mm plates works well). Monolayers are used for assays within 24-48 hours after reaching confluency.

Virus used for infections is diluted in Tris-Saline + 2% fetal calf serum. The infections are carried out for 1-2 hours in a 37°, CO_2 incubator with occasional rocking of the plates.

SV40 DNA infections are done at room temperature for 15-20 mins. using Pagano's procedure. The DNA to be used is diluted in Tris-Saline and mixed 1:1 (v/v) with DEAE-Dextran ($MW \sim 2 \times 10^6$) to give a final DEAE-Dextran concentration of about 500 µg/ml. It is important to thoroughly (i.e. 2 times) wash the monolayers with Tris-Saline before and after infection.

The initial agar overlay of 5 mls. per plate consists of 1 x AutoPow MEM, 4-5% fetal calf serum, 1.0% Difco Bacto-Agar, and penicillin and streptomycin.

At about 5 days p.i., a second overlay of 3 mls. per plate is added. This overlay is the same as before except that 1% rather than 4-5% fetal calf serum is used.

Clearly visible plaques first appear at about 7 days p.i.. The plaques are seen as spots in the monolayer that are "whiter" than the rest of the monolayer to the naked eye, but appear as regions of piled up cells under a microscope.

At about 9 days p.i., an additional 3 ml overlay (this time including 0.01% neutral red) is added. The "white spots" of before will now appear as minute plaques that get larger with time. Plaques are counted for several days starting at day 10.

With isolated Form I SV40 DNA the efficiency of plaque formation is $\sim 10^7$ pfu/µg DNA.